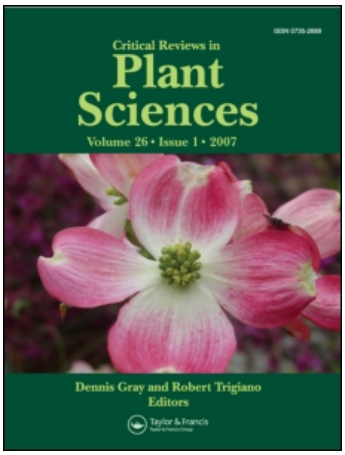

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Poplar and its Bacterial Endophytes: Coexistence and Harmony

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Associations between plants and microorganisms are very complex and are the subject of an increasing number of studies. Here, we specifically address the relationship between poplar and its endophytic bacteria. The role and importance of endophytic bacteria in growth and development of their host plants is still underestimated. However, since many endophytes have a beneficial effect on their host, an improved understanding of the interaction between poplar and its endophytic bacteria has the potential to provide major breakthroughs that will improve the productivity of poplar.

Endophytic bacteria can improve plant growth and development in a direct or indirect way. Direct plant growth promoting mechanisms may involve nitrogen fixation, production of plant growth regulators such as auxins, cytokinins and gibberellins, and suppression of stress ethylene synthesis by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. Endophytic bacteria can indirectly benefit the plant by preventing the growth or activity of plant pathogens through competition for space and nutrients, antibiosis, production of hydrolytic enzymes, inhibition of pathogen-produced enzymes or toxins, and through systemic induction of plant defense mechanisms.

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Examples of applications for custom endophyte-host partnerships include improved productivity and establishment of poplar trees on marginal soils and the phytoremediation of contaminated soils and groundwater. A systems biology approach to understand the synergistic interactions between poplar and its beneficial endophytic bacteria represents an important field of research, which is facilitated by the recent sequencing of the genomes of poplar and several of its endophytic bacteria.

Keywords poplar, endophytic bacteria, plant growth promoting bacteria, phytohormones, biomass production, phytoremediation, carbon sequestration

I. INTRODUCTION

The varied and complex associations between plants and microorganisms have been the subject of considerable research and diverse applications (Lodewyckx *et al.*, 2002; Mastretta *et al.*, 2006). Endophytic bacteria can be defined as bacteria that reside within living plant tissue without causing substantive harm to their host. A diverse array of bacterial genera have been reported to be endophytic [see (Mastretta *et al.*, 2006) for recent review]. A close relationship between endophytic and rhizosphere bacteria exists, and many facultative endophytic bacteria can also survive in the rhizosphere.

A general effect of plant-microbe interactions is an increase of microbial biomass and activity in the rhizosphere as compared to bulk soil (Merckx *et al.*, 1986). Studies have revealed that bacterial densities are highest in the rhizosphere and decrease progressively from the roots to the stem and the leaves where bacterial density is lowest (Lamb *et al.*, 1996; QuadtHallmann and Kloepper, 1996; Porteous Moore *et al.*, 2006). Bacterial entry into plants predominantly occurs via the roots, more precisely at sites of epidermal/exodermal damage, that naturally arise due to development of lateral roots, through root hairs or at epidermal conjunctions (Sprent and Defaria, 1988). Once inside the plant, endophytic bacteria either colonize the plant systematically by migration through the vascular system or the apoplast, or remain localized in a specific plant tissue like the root cortex or the xylem, (Hurek *et al.*, 1994; James *et al.*, 1994; Mahaffee and Kloepper, 1997b, a; QuadtHallmann *et al.*, 1997). Established endophytic communities receive nutrients from the host plant and in exchange can improve plant growth and health, either directly or indirectly.

This review discusses the association between poplar and its endophytic bacteria, the mode of colonisation and highlights two examples of the beneficial effects that endophytes can have on plant growth and development.

II. ENDOPHYTIC BACTERIA ASSOCIATED WITH POPLAR

Since poplar is an important tree species for the phytoremediation of contaminated groundwater, earlier work on endophytic bacteria from poplar focused on the isolation and characterization of cultivable endophytes that were able to complement the metabolic properties of their host plant.

Van Aken *et al.* (Van Aken B., 2004a) isolated an endophytic strain, *Methylobacterium populi* BJ001, from poplar (*Populus deltoids* x *nigra* DN34). *M. populi* BJ001 is a facultative methylotrophic bacterium belonging to the alpha-proteobacteria. Methanotrophs belong to a group of bacteria that utilize methanol and methylamine as their sole source of carbon and energy. Within this group, there is a great deal of diversity in their carbon utilization efficiencies, in their nitrogen fixation abilities, and in their abilities to oxidize trichloroethylene (TCE), a common groundwater contaminant. From a metabolic point of view, *M. populi* BJ001 is especially interesting as this strain is able to mineralize different nitro-substituted explosives (trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine or Royal Demolition Explosive (RDX) and 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX)) to CO_2 (Van Aken B., 2004b), as well as methyl-t-butyl ether (MtBE) (van der Lelie and Moreels, unpublished results). Since *Methylobacterium* represents a group of microorganisms commonly found as endophytes in a variety of plant species, their involvement in phytoremediation of a broad spectrum of organic contaminants may be of significant importance.

The diversity of endophytic bacteria found in association with poplar was investigated as part of a larger study to assess the possibility and practicality of using endophytic bacteria to enhance *in situ* phytoremediation (Porteous Moore *et al.*, 2006; Barac *et al.*, 2009). Endophytic bacteria were isolated from the root, stem and leaf of two cultivars of poplar (*Populus trichocarpa* x *P. deltoides* cv. "Hazendans" and "Hoogvorst") growing on a site where they were used to contain a groundwater plume contaminated with benzene, toluene, ethylbenzene and xylene (BTEX) compounds. One hundred twenty-one morphologically distinct cultivable isolates were obtained, belonging to 21 genera, although 6 isolates could not be identified with confidence to the genus level. Bacteria of the gamma-proteobacteria dominated the collection of isolates, comprising 59% of the total, including 42% *Pseudomonas* spp., with smaller percentage numbers of *Xanthomonas* spp., *Acinetobacter* spp. and *Enterobacter* spp. representing the majority of the remainder of the gamma-proteobacteria. The beta-proteobacteria made up 18% of the isolate collection, with *Burkholderia* spp. (10%) and *Herbaspirillum* spp. (4%) representing the majority of the group. The alpha-proteobacteria formed 10% of the total number of isolates and were largely represented by *Sphingomonas* spp. (9%). Gram-positive bacteria comprised 13% of the total number of isolates, represented largely by *Arthrobacter* spp. (10%), *Bacillus* spp., *Paenibacillus* spp., and *Agreia* spp. Interestingly, the endophytic bacteria exhibited marked spatial compartmentalization within the plant (Porteous Moore *et al.*, 2006). A number of isolates showed the ability to degrade BTEX compounds or to grow in the presence of TCE, demonstrating that within the diverse bacterial

communities found in poplar several endophytic strains are present that have the potential to enhance phytoremediation strategies (Porteous Moore *et al.*, 2006; Barac *et al.*, 2009). When the genome of *P. trichocarpa* was sequenced [see http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html; (Tuskan *et al.*, 2006)], a variety of putative endophyte sequences were also identified (S. DiFazio, pers. comm.). These included some members of these same genera as well as *Bradyrhizobium*, *Sinorhizobium*, *Ralstonia* and *Rhodobacter*.

The dominance of the gamma-proteobacteria among the endophytes from poplar was repeatedly observed (Taghavi *et al.*, 2009). Among 78 endophytic bacteria isolated from surface sterilized root and stem samples taken from hybrid poplar H11–11 (*Populus trichocarpa* x *P. deltoides*) and native willow (*Salix gooddingii*) that were grown in the presence of TCE for over five years, the majority of the isolated strains (71%) belonged to the gamma-proteobacteria with *Serratia* spp., *Serratia plymuthica*, *Serratia proteamaculans* and *Rahnella* spp. being the most frequently found. Other dominant gamma-proteobacteria included *Pseudomonas* spp. and *Enterobacter* spp. The Actinobacteria (15% of the population) were dominated by *Rhodococcus* spp.

The community structure of endophytic bacteria was shown to be strongly affected by different hybrid poplar clones (Ulrich *et al.*, 2008a), pointing to species-specific associations between endophytes and their poplar host. Detailed analysis of endophytic bacteria from different hybrid poplar clones revealed a high phylogenetic diversity of endophytic bacteria with a total of 53 taxa at the genus level that included proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes. Interestingly, the community structure displayed clear differences in terms of the presence and relative proportions of bacterial taxa between the four poplar clones studied, and corresponded well with the genetic background of the hybrid poplar clones (Ulrich *et al.*, 2008a). Cultivation conditions also influence the composition of the endophytic community. After 5 years of micro-propagation, the endophytic communities associated with poplar, larch and spruce were dominated by bacteria that could be assigned to the genus *Paenibacillus* (Ulrich *et al.*, 2008b). Other endophytic bacteria such as *Methylobacterium*, *Stenotrophomonas* or *Bacillus* were also found but only in some tissue cultures. (Ulrich *et al.*, 2008b).

Poplar roots often extend beyond soil into groundwater and have access to bacteria typical of oligotrophic water habitats (including the ubiquitous proteobacteria), which may be ecologically distinct from those found in higher nutrient soils. Data reported from filtered and chlorine treated drinking water distribution systems (oligotrophic water) have documented the presence of a diverse bacterial community in biofilms and in bulk water (LeChevallier *et al.*, 1996; Lerat *et al.*, 2003; Lehtola *et al.*, 2004; Lerat *et al.*, 2005). Whether or not bacteria in oligotrophic groundwater (including proteobacteria) will be able to form associations with poplar remains to be confirmed. Overall, the fact that many endophytes that were found in poplar growing in contaminated environments (e.g. BTEX, MtBE, TCE) are members of taxa that make up the second largest group of bacteria, i.e., the gram negative gamma-protobacteria, indicates that there is very good potential for the development of improved phytoremediation strategies based on plant-microbe interactions.

III. COLONIZATION BY ENDOPHYTIC BACTERIA

A close relationship exists between endophytic and rhizosphere bacteria and many endophytic bacteria can enter their host plant via the roots. This concept was successfully applied to inoculate poplar cuttings with endophytic bacteria (Germaine *et al.*, 2004; Taghavi *et al.*, 2005; Taghavi *et al.*, 2009; Weyens *et al.*, 2009). Root colonization by rhizosphere bacteria can be considered to involve several stages (Brimecombe *et al.*, 2007), and a similar process is expected to happen with endophytic bacteria. In the initial stage bacteria move to the plant roots, either passive via soil water fluxes or active via specific induction of flagellar activity by plant-released compounds (chemotaxis). In a second step, nonspecific adsorption of bacteria to the roots takes place, which is followed by anchoring (third step), resulting in the firm attachment of bacteria to the root surface. Specific or complex interactions between the bacterium and the host plant, including the secretion of root exudates, may arise that can result in the induction of bacterial gene expression (fourth step). Endophytic bacteria can subsequently (fifth step) enter their host plant at sites of tissue damage, which naturally arise as the result of plant growth (lateral root formation), or through root hairs and at epidermal conjunctions (Sprent and Defaria, 1988). In addition, plant exudates leaking through these wounds provide a nutrients source for the colonizing bacteria and hence create favorable conditions. This root colonization strategy was confirmed by several microscopic studies (Wiehe *et al.*, 1994; Benhamou *et al.*, 1996a, b; Pan *et al.*, 1997), including poplar (Germaine *et al.*, 2004). Alternatively, endophytic bacteria can use vector organisms (e.g. arbuscular mycorrhizae and insects) to gain entrance to the apoplastic spaces to colonize the host plant (Ashbolt and Inkerman, 1990; Kluepfel, 1993; Franke *et al.*, 2000). Although likely to occur for many plant species, the involvement of specific vector organisms for endophytic colonization has not been demonstrated to play a role in poplar. In some plant species, the importance of seed endophytes as a vector for beneficial bacteria has been demonstrated (Cankar *et al.*, 2005; Mastretta *et al.*, 2009), including tobacco where seed endophytes had a beneficial effect on metal toxicity and translocation (Mastretta *et al.*, 2009). Since poplar is multiplied by cutting, this way of transferring endophytes is not relevant for the practical application of this species.

Although the colonization of plants by endophytes has already been established, the colonization patterns and relative density of a particular endophyte is not well understood in poplar (Germaine *et al.*, 2004; Taghavi *et al.*, 2005; Taghavi *et al.*, 2009; Weyens *et al.*, 2009). To study the colonization patterns and population sizes of bacterial endophytes in poplar,

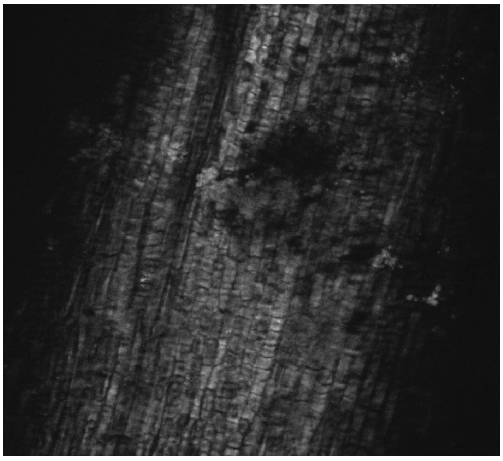

FIG. 1. Colonization of the surface of a poplar root by *gfp*-labeled derivative of the endophyte *P. putida* W619. The picture was taken by fluorescence microscopy.

endophytic bacteria were isolated from xylem sap of poplar (*Populus trichocarpa* x *P. deltoides* cv. Hoogvorst) (Germaine *et al.*, 2004). After their identification, three *Pseudomonas* spp. were *gfp*-labeled and re-introduced into their host plant. Two of the endophytes showed considerable colonization of the poplar roots and stems, and *gfp* expressing cells of all three strains were observed to colonize the xylem tissue of the root. All three strains also proved to be efficient rhizosphere colonizers, supporting the theory that the rhizosphere can serve as a source of bacterial endophytes (Germaine *et al.*, 2004). However, *gfp* expression might have negative effects on endophytic colonization and growth and development of poplar. Inoculation of poplar cuttings [*Populus deltoides* x (*P. trichocarpa* x *P. deltoides*) cv. Grimminge] with the endophytic strain *Pseudomonas putida* W619 (Taghavi *et al.*, 2005; Taghavi *et al.*, 2009) (wild-type or *gfp*-labeled) showed that the *gfp*-labelled strains only colonized the rhizosphere and root cortex (Fig. 1) while the wild-type strain also colonized the xylem vessels of the root (Weyens *et al.*, 2009). Analysis of morphological, physiological and biochemical parameters of the inoculated poplar cuttings showed that inoculation with the *gfp*-labelled W619 strains had a negative effect on plant fitness, shown by a negative effect on plant growth, increased superoxide dismutase activity in the roots, and a significant decrease in stomatal conductance. Since in both strains the *gfp*-transposon did not insert in areas coding for genes that could be presumed to be involved in colonization or phytohormone balance, the observed differences after inoculation between the wild-type and *gfp*-labelled strains might be related to *gfp* expression, probably causing a 'stress effect' on the plant cells, more specifically on root cells, leading to an inhibition of root development and a generally decreased plant fitness (Weyens *et al.*, 2009).

Analysis of the genome of *Enterobacter* sp. 638, a plant growth promoting endophyte from poplar (Taghavi *et al.*, 2009) whose genome was sequenced by the Joint Genome Institute (http://genome.jgi-psf.org/ent_6/ent_6.home.html), further supports a multi step root colonization and entry process. This analysis revealed the presence of several gene clusters important for cell mobility including four flagellar biosynthesis operons (*FlgNMABCDEFGHIJKL*, *flhEAB fimA yraIJ lpfD cheZYBR tap tar csuEDCAB int cheWA motBA flhCD, fliYZA fliCDSTEFGHJKLMNOPQR* and *fliEFHIJKLMNOPQR*), which are very similar to those found in *Salmonella enterica* subsp. *Enterica typhi* and *E. coli* K12 (Chilcott and Hughes, 2000), except that the *flh* operon of *Enterobacter* sp. 638 contains two insertions of pili biosynthesis genes. In addition, the *Enterobacter* sp. 638 genome contains a number of genes associated with agglutination and cell adhesion, similar to those found in both animal and plant pathogens. Many of these genes are not present in *E. coli* K12, and are hypothesized to be important for plant colonization (Monchy and van der Lelie, unpublished results). The genes include filamentous haemagglutinin (which is implicated in cell aggregation as a surface-exposed and secreted protein, and acts as a major virulence attachment factor) (Relman *et al.*, 1989; Cotter *et al.*, 1998), autotransporters with a pertactin or hemagglutinin domain (which are adhesins that are exported via the autotransporter protein mechanism; (Henderson *et al.*, 1998), and virulence factors (such as those encoded by the *srfABC* operon (Worley *et al.*, 2000) located on both the chromosome and plasmid pENT638–1).

The 157.7 kb plasmid pENT638–1 of *Enterobacter* sp. 638 is related to F-plasmids found in other *Enterobacteriaceae*. Plasmids of this family are involved in host interaction and virulence, such as the pFra plasmid of *Yersinia pestis* (Golubov *et al.*, 2004). In pENT638-1 the pFra pathogenicity island has been replaced by a 23kb putative genomic island (flanked by an integrase gene, and having a GC% significantly different that the rest of the plasmid), which contains a group of ORFs with strong homology to hypothetical proteins of *Azotobacter vinelandii* AvOP, as well as a putative *srfABC* operon, which is also present in a horizontally acquired region of *Salmonella* spp. and is believed to be involved in virulence (Worley *et al.*, 2000). Adjacent to this region, a putative *ndvB* (8532 bp) gene is located. NdvB, which in involved in the production of beta-(1->2)–glucan, is required by *Sinorhizobium meliloti* for bacterial invasion of nodules (Dylan *et al.*, 1986). Many other genes involved in plant invasion were present on pENT638-1, coding for proteins with an autotransporter domain (secretion type V) of virulence domains (agglutinin, pertactin or adhesin). Quantitative PCR showed a 4.5 fold induction of the nvdB gene after 2 hours incubation of *Enterobacter* sp. 638 in the presence of poplar roots (Monchy and van der Lelie, unpublished results). Other induced functions included an autotransporter protein with a pertactin domain (Ent4206; 4.5-fold induction) and an autotransporter with a hema-agglutinin domain (Ent4267; 4.5-fold induction), both presumably involved in cell adhesion and root colonization.

IV. PLANT GROWTH PROMOTING MECHANISMS

Endophytic bacteria have several direct and indirect mechanisms by which they can promote plant growth and health. Understanding these mechanisms will enhance the value of poplar and other plants as feedstocks for biofuel production, particularly on marginal soils where biofuel crops will not displace food production from arable land.

Direct plant growth promoting mechanisms from endophytic bacteria may involve nitrogen fixation (James, 2000; Doty, 2008), the production of plant growth regulators such as auxins, cytokinins and gibberellins (Bent, 2001; de Salamone *et al.*, 2001; Asghar *et al.*, 2004), suppression of the production of stress ethylene by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Belimov, 2005; Dell'Amico *et al.*, 2005) and alteration of sugar sensing mechanisms in plants (Goddijn and Smeekens, 1998). Trehalose, a nonreducing disaccharide, is the principal storage carbohydrate of bacteria, yet it can be produced in plants though to a much lesser extent than sucrose (Paul et al 2008). Even so, this sugar is thought to play a pivotal role in plants controlling their partitioning of carbon (Ramon and Rolland, 2007) especially into cell wall biomass (Gomez *et al.*, 2006). Furthermore, activity levels of trehalase, the principal enzyme responsible for degrading this sugar, were shown to be strongly induced by infection with the trehalose-producing pathogen *Plasmodiophora brassicase* (Brodmann *et al.*, 2002). Alteration of biosynthesis and/or metabolism of trehalose *in planta* has been shown to increase tolerance to drought, salt, and cold (Garg *et al.*, 2002). It is therefore noteworthy that several endophytic bacteria from poplar were able to efficiently metabolize trehalose (Taghavi *et al.*, 2009).

Plant-associated bacteria can also indirectly benefit plant growth by preventing the growth or activity of plant pathogens through competition for space and nutrients (Buyer and Leong, 1986; Buyer *et al.*, 1986; O'Sullivan and O'Gara, 1992), antibiosis (Dowling and O'Gara, 1994; Ramos-Gonzalez *et al.*, 2005), production of hydrolytic enzymes (Krechel *et al.*, 2002), inhibition of pathogen-produced enzymes or toxins (Bertagnolli *et al.*, 1996) and through induction of plant defense mechanisms (van Loon *et al.*, 1998; Spencer *et al.*, 2003; Jeun *et al.*, 2004; Kloepper *et al.*, 2004; Ryu *et al.*, 2004; Zhang *et al.*, 2004). Recently, a *Rhizobium tropici* strain that lives within the stems of poplar has been discovered (Doty *et al.*, 2005). This diazotrophic (nitrogen-fixing) species is known for its ability to nodulate an exceptionally wide range of legumes; however, its endophytic nature in non-legume species has not been described previously (Doty *et al.*, 2005), and its role in providing nitrogen to poplar is unclear.

Short-term beneficial effects of plant growth promoting microorganisms, observed during the first weeks or months, will specifically impact plant growth and establishment. These effects include accelerated root development resulting in better access to nutrients and water, and consequently a faster initial growth, which will allow the plants to out compete weeds for available resources. This will allow for the improvement of the establishment of poplar on marginal soils, and reduce the need for synthetic fertilizers, intensive irrigation or application of high doses of herbicides. Long-term beneficial effects of plant growth promoting microorganisms, which can be observed over several growth seasons, may result in improved plant growth, health and survival, leading to economically sustainable feedstock production. This may be obtained by counteracting stress responses caused by drought and contamination, protection against pathogens via competition for available resources, and by assisting the plant's defense response against pathogenic invasions. So far, most studies have addressed the short-term beneficial effects of endophytic bacteria on the growth and development of poplar. These studies have ranged from days to several weeks. For instance, poplar cuttings (*Populus deltoides* x *P. nigra* DN-34) that were allowed to root in the presence of endophytic bacteria, such as *Serratia proteamaculans* 568, *Enterobacter* sp. 638 and *P. putida* W619 showed significantly improved root and shoot formation when grown in hydroponics (Taghavi *et al.*, 2009). In contrast, root formation was very slow for non-inoculated plants.

From the poplar derived endophytic bacteria tested for their short-term plant growth promoting properties, *Enterobacter* sp. 638 had the most pronounced beneficial effect on the development and growth of poplar cuttings when planted in marginal soil (Fig. 2). This result was repeatable with *P. deltoides* x *P. nigra* DN-34, as well as with the hybrid poplar clone OP367 (*Populus deltoides* x *P. nigra*) (significance level: $p < 0.05$; L. Newman, unpublished results). After 10 weeks of growth, poplar inoculated with *M. populi* BJ001 had less new biomass than the controls (p value < 0.05). On the other hand, while no significant plant growth promoting effect was observed for *P. putida* W619 with *P. deltoides* x *P. nigra* DN-34, strain W619 significantly (significance level: $p < 0.01$) promoted the growth of another hybrid poplar (*Populus deltoides* x (*trichocarpa* x *deltoides*) cv. Grimminge) (Weyens *et al.*, 2009). Also, the promiscuous plant growth promoting effect of *B. cepacia* Bu72 on poplar DN-34 (Taghavi *et al.*, 2005; Taghavi *et al.*, 2009) and yellow lupine (Barac *et al.*, 2004) is noticeable. Therefore, before applying plant growth promoting endophytic bacteria to other poplar cultivars to promote their short-term growth and establishment, preliminary studies to confirm the plant growth promoting synergy of the selected endophytes and selected poplar clones is required. This is also consistent with the observed differences in endophytic community structure between hybrid poplar clones (Ulrich *et al.*, 2008a).

Recently, studies using the short-lived radioisotope ^{11}C to image whole-plant allocation of radiolabeled photosynthate (Ferrieri *et al.*, 2005; Ferrieri *et al.*, 2006; Thorpe *et al.*, 2007) revealed new insights into the short term responses of the hybrid poplar clone OP367 (*Populus deltoides* x *P. nigra*) to colonization by different soil and endophytic bacteria (Barac and Ferrieri, unpublished). Specifically, plants responded with elevated rates of

FIG. 2. Effect of plant growth promoting endophytic bacteria on the growth of poplar on marginal soils. Selected cultivable endophytic γ-proteobacteria, found in poplar, are tested for their capacity to improve growth of their host plant. Representative plants are shown that were inoculated with *M. populi* BJ001 (plant 1), *S. maltophilia* R551-3 (plant 4), *P. putida* W619 (plant 5), *S. proteamaculans* 568 (plant 6), *Enterobacter* sp. R558-1 and *Enterobacter* sp. 638 (plant 8). *Burkholderia cepacia* BU72, an endophyte originally isolated from yellow lupine which was found to have plant growth promoting effects on poplar (Taghavi *et al.*, 2005), and *Cupriavidus metallidurans* CH34 (also referred to as *Ralstonia metallidurans* CH34) (Monchy, 2006) a typical soil bacterium with no known plant growth promoting effect, were included as positive and neutral controls (plant 9 and 2, respectively). Also, non-inoculated cuttings (plant 3) were used as controls. Plants were grown for 10 weeks on a marginal sandy soil under greenhouse conditions.

leaf export of ^{11}C-photosynthate 3 days after inoculation using *B. cepacia* (BU61) a soil bacteria, and *Burkholderia cepacia* (VM1468) and *Pseudomonas putida* (W619), both endophytic bacteria. Furthermore, BU61 and VM1468 showed evidence of increased apical partitioning of plant carbon relative to control plants (significance level: $p < 0.05$).

In addition to the sequencing previously described for *Enterobacter* 638, several endophytic bacteria, representing the dominant genera of endophytic gamma-proteobacteria found in poplar, were selected for genome sequencing and analysis of their plant growth promoting effects, including root development (Taghavi *et al.*, 2009). Genome sequencing was carried out at the Joint Genome Institute (DOE, Walnut Creek, CA) on *P. putida* W619 (genome available at http://genome.jgi-psf.org/psepw/psepw.home.html), *Serratia proteamaculans* 568 (genome available at http://genome.jgi-psf.org/serpr/serpr.home.html) and *Stenotrophomonas maltophilia* R551-3 (genome available at http://genome.jgi-psf.org/stema/stema.home.html).

The growth promoting effect of *Enterobacter* sp. 638 on poplar might be explained by the presence of the putative *als*DS pathway for acetoin synthesis, a potent plant growth promoting compound (Ryu *et al.*, 2003; Ping and Boland, 2004). As with the rhizosphere bacterium *Bacillus amyloliquefaciens* FZB42 (Chen *et al.*, 2007) it was unclear which function catalyzes the conversion of acetoin into 2,3-butanediol, a compound that can induce systemic tolerance to drought in *Arabidopsis thaliana* (Cho *et al.*, 2008) and systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco (Han *et al.*, 2006). *Enterobacter* sp. 638 also possesses a putative acetoin reductase for synthesis of diacetyl, which role in plant growth promotion is unknown. None of the other traits typically linked to plant growth regulation were identified in *Enterobacter* sp. 638: the strain produces low levels of indole acetic acid (IAA), is unable to fix nitrogen, and lacks the pathways to metabolize ACC, gamma-amino-butyric acid (GABA) and phenyl acetic acid (PAA), two compounds involved in regulating plant responses to stress.

S. proteamaculans 568 is interesting as it is, in contrast to *Enterobacter* sp. 638, able to metabolize GABA and PAA. This strain has the genetic pathway to produce 2-acetoin, but lacks the acetoin reductase for the bidirectional conversion of acetoin and diacetyl. Furthermore, it lacks the putative plant invasion functions found on plasmid pENT628–1.

P. putida W619 seems to be well adapted to influence the phytohormone balances of its host: the strain appears to produce high levels of IAA and is able to metabolize PAA and GABA. Elevated levels of GABA and PAA, a non-indolic auxin that can account for up to one-half of the total bio-assayable auxin activity in plant extracts (Wightman, 1975), can inhibit plant growth. The complexity of the phytohormone balance points towards the existence of a complex mechanism that fine-tunes the interaction between *P. putida* W619 and other endophytes and their poplar host. For instance, the negative effects on poplar development observed after inoculation with *M. populi* BJ001 might reflect a disturbance of this balance, e.g., caused by unnaturally high numbers of this bacterium during inoculation.

No data are available on the long term effects of inoculation of poplar with beneficial endophytic bacteria. However, analysis of the *S. proteamaculans* 568, *Enterobacter* sp. 638, *S. maltophilia* R551-3 and *P. putida* W619 genomes revealed the presence of several putative functions via which these endophytes could have a lasting beneficial effect on poplar. For instance, all four strains are capable of producing one or several siderophores that can be used to compete with pathogenic microorganisms for available ferrous iron [Fe(III)] (Table 1). In addition, the strains are capable of taking up and metabolizing a large spectrum of heterologously produced siderophores, thus

TABLE 1
Predicted number of genes coding for proteins involved in iron uptake in endophytic bacteria, isolated from poplar, whose genomes have been sequenced

	E. coli K12	Enterobacter 638	S. proteamaculans 568	P. putida W619	S. maltophilia R551-3	S. maltophilia K279a
Number genes coding for an ABC transporter component	209 genes	285 genes	339 genes	225 genes	67 genes	61 genes
Putative Iron uptake ABC transporter	4 operons	9 operons	7 operons	5 operons	—	—
TonB-dependent receptors	7 genes	12 genes	3 genes	16 genes	81 genes	65 genes
Ferric siderophore uptake	*1 operon exbDB*	*1 operon exbDB*	*2 operons exbDB*	*3 operons exbDB*	*3 operons exbDB + exbD*	*3 operons exbDB*
Ferrous iron uptake	*feoAB, efeBOU*	*feoAB, efeBOU*	*feoAB, efeBOU*	—	*feoAB*	feoAB
Ferritin (iron storage)	2 operons	2 operons	1 gene	4 genes (*bfd-bfrB*, *bfr*, *bfrA*)	5 genes (*bfrA*, *bfr*, *dps*, *bfr*, *bfd*)	5 genes (*bfrA*, *bfr*, *dps*, *bfr*, *bfd*)
Enterobactin	13 genes	17 genes	12 genes	1 gene	6 genes	6 genes
Biosynthesis (Ent)	*entSABECFD*	*entSABECFD*	*entABCEFS*	—	*entAFBEC*	*entAFBEC*
Uptake (Fep, Sit)	*fepACGDB*	*fepACGDB, sitABCD*	*fepABCDG*	*fepA*	—	—
Release iron from enterobactin	*fes*	*fes*	*fes*	—	*fes*	*fes*
Aerobactin	—	—	6 genes	—	—	—
TonB-dependent ferric aerobactin receptor	—	—	*iutA*	—	—	—
Aerobactin siderophore biosynthesis	—	—	*iucDCBA*	—	—	—
Siderophore interacting protein	—	—	Spro_0924	—	—	—
Pyoverdine	—	—	*3 genes*	7 genes	—	—
Pyoverdine biosynthesis	—	—	*pvcAB*	*pvsA*, *pvdZ*, *pfrI*	—	—
Putative pyoverdine sidechain peptide synthetase	—	—	—	*PputW6193541-3542, 3546*	—	—
Pyoverdine ABC transporter	—	—	—	*pvdE*	—	—
Ferripyoverdin TonB-dependent receptor	—	—	*fpvA*	—	—	—
Non-ribosomal siderophore biosynthesis	—	—	Spro_0974-0978	—	—	—

The number of genes, predicted by manual annotation, that code for proteins involved in iron uptake are presented for the poplar endophytes *Enterobacter* 638, *S. proteamaculans* 568, *P. putida* W619 and *S. maltophilia* R551-3, and for selected closely related non-endophytic bacteria, *E. coli* K12 and *S. maltophilia* K279a. The table displays the total number of genes coding for ABC transporters, and the subgroup of complete ABC transporter operons putatively involved in iron uptake. The genes coding for TonB-dependant receptors, most of which are putatively involved in iron uptake, ferric siderophore uptake systems, ferrous iron uptake systems, and iron storage systems were identified. In addition operons for the synthesis and processing of enterobactin, aerobactin, pyoverdine and an undefined non-ribosomal siderophore were identified among the analyzed bacteria. For each siderophore operon, the genes were classified according to their putative functions: siderophore biosynthesis, siderophore export, ferri-siderophore uptake and the release of iron from the iron-siderophore complex in the cytoplasm.

increasing their competitiveness for ferrous iron, which is often a major microbial growth limiting factor. For example, *S. maltophilia* R551-3 encodes several transporter proteins involved in iron uptake, including siderophore receptors for ferric-alcaligin and ferrichrome. Ferrichrome has been found to be produced by fungi of the several genera including phytopathogenic fungi of the genus *Ustilago* (Ardon *et al.*, 1997). Fungal growth inhibiting bacteria, such as *P. putida*, are often able to utilize heterologously produced ferrichrome (Jurkevitch *et al.*, 1992). It is therefore also expected that *S. maltophilia* R551-3 may control the growth of fungal pathogens and other pathogenic microorganisms via competition for iron.

Mannitol, an effective scavenger of reactive oxygen, is produced by species of pathogenic fungi as a way to counteract the plant defense that involves the production of reactive oxygen species (Jennings *et al.*, 1998). Mannitol dehydrogenase expression can enhance plant resistance to mannitol-secreting fungal pathogens (Jennings *et al.*, 2002). Mannitol can not be metabolized by poplar, but several poplar endophytes, including *S. proteamaculans* 568, *Enterobacter* sp. 638, *S. maltophilia* R551-3 and *P. putida* W619 can grow on mannitol as sole carbon source (Taghavi *et al.*, 2009). Therefore these endophytes can possibly assist their poplar host in its defense against mannitol-secreting pathogenic fungi by metabolizing mannitol (Monchy and van der Lelie, unpublished). Bacterial chitinases are very effective to hinder the growth and development of pathogenic fungi. *S. maltophilia* R551-3 and *P. putida* W619 are able to efficiently degrade and grow on chitine and might protect poplar against fungal infections, thus providing an indirect benefit for the growth of poplar. Finally, antibiotic production and resistance represents another antagonistic mechanism present in *S. maltophilia* R551-3. This strain produces the antifungal macrocyclic lactam antibiotic maltophilin (β-lactamase L1) that efficiently inhibit the growth of various saprophytic, zoo-pathogenic and phytopathogenic fungi (Jakobi *et al.*, 1996). Overall, bacteria from the genus *Stenotrophomonas* (Messiha *et al.*, 2007) and *Pseudomonas* (Barka *et al.*, 2002) are known to be excellent antagonists with the potential to interfere with pathogen infection, growth, and survival. The beneficial properties of *S. maltophilia* R551-3 were recently reviewed and compared to that of the opportunistic pathogen *S. maltophilia* K279a (Ryan *et al.*, 2009).

V. ENDOPHYTE-ASSISTED PHYTOREMEDIATION

Poplar has been very successfully used for the remediation of groundwater contaminants such as TCE, MTBE and BTEX compounds. Early work by several groups (Muralidharan *et al.*, 1995; Schnoor *et al.*, 1995; Newman *et al.*, 1997; Doucete *et al.*, 1998; Gordon *et al.*, 1998; Newman *et al.*, 1999; Dietz and Schnoor, 2001; Rubin and Ramaswami, 2001) showed that plants were able to take up and degrade water soluble compounds into nontoxic or less-toxic metabolites. This included several varieties of poplar, willow, eucalyptus, sycamore, sweet gum, pine and other deep-rooted plants, all of which showed degradation abilities. Analysis of the cultivable microbial communities associated with poplar growing on a BTEX contaminated ground water plume demonstrated that, once the poplar roots got in contact with the BTEX contaminated groundwater, enrichment occurred of both rhizosphere and endophytic bacteria that were able to degrade toluene (Barac *et al.*, 2009). Interestingly, once the BTEX plume was remediated, the numbers of cultivable toluene degrading rhizosphere and endophytic bacteria decreased below the detection limit, indicating that their population resulted from selective enrichment by the presence of the contaminants. However, depending on the concentration of the parent compound in the aquifer, the percent that is degraded by the plant and/or associated endophytes versus the percent that is volatilized by the plant (Vroblesky *et al.*, 1999; Ma and Burken, 2002; Arnold *et al.*, 2007) might impact the acceptability of phytoremediation in areas that have a no-emission policy for remediation technologies.

The fate of contaminants in the rhizosphere-root system largely depends on its physicochemical properties. Organic xenobiotics with a log K_{ow} (octanol-water partition coefficient) <1 are considered to be very water-soluble, and plant roots do not generally accumulate them at a rate surpassing passive influx into the transpiration stream (Cunningham and Berti, 1993; Cunningham *et al.*, 1995). Contaminants with a log K_{ow} >3.5 show high sorption to the roots, but slow or no translocation to the stems and leaves (Trapp *et al.*, 2001). However, plants readily take up organic xenobiotics with a log K_{ow} between 0.5–3.5, as well as weak electrolytes (weak acids and bases). These compounds seem to enter the xylem faster than the soil and rhizosphere microflora can degrade them, even if the latter is enriched with degrader bacteria (Trapp *et al.*, 2000). Once taken up, plants and/or endophytic bacteria metabolize contaminants, such as TCE, to predicted oxidative metabolites such as trichloroacetic acid (TCAA) (Newman *et al.*, 1997; Doucete *et al.*, 1998). The levels of TCAA in plants are not toxic to the plant or animals that feed on the plants. Alternatively, plants release volatile compounds into the environment by evaporation via the leaves or trunk (Burken and Schnoor, 1996; van der Lelie *et al.*, 2001; Schroder *et al.*, 2002; Ma and Burken, 2003; Burken *et al.*, 2005).

Acceptance of phytoremediation of volatile and water soluble organic xenobiotics may be improved by using recombinant endophytic bacteria modified to contain the appropriate degradation pathway (Barac *et al.*, 2004). Endophytic bacteria equipped with a toluene degradation pathway were able to reduce toluene phytotoxicity and evapotranspiration from their yellow lupine host plant. Although the application of engineered endophytic bacteria to improve phytoremediation of volatile organic contaminants has several obvious advantages over the application of engineered rhizosphere bacteria or the genetic engineering of the plant's metabolism, several obstacles need to be overcome before this technology can move towards application (Newman and Reynolds, 2005). One major point of concern is the persistence and the stability of the engineered organisms

and their degradation capabilities in field grown plants, as phytoremediation projects can conceivably last decades. As long as a selection pressure is present, there will be an advantage for those endophytic community members possessing the appropriate degradation characteristics (Barac *et al.*, 2009), but there is no guarantee that the inoculum will become an integrated part of the endogenous endophytic community.

Many pathways for the degradation of organic contaminants are located on mobile, elements, including plasmids and transposable elements. Therefore, it was hypothesized that horizontal gene transfer of these mobile elements plays an important role in adapting the endogenous endophytic community (van der Lelie *et al.*, 2005): rather than integrating a new bacterium in a stable community, the degradation pathway is transferred among the members of the community. This was first demonstrated by inoculating *Populus trichocarpa* x *deltoides* cv. "Hoogvorst" with the endophytic strain *Burkholderia cepacia* VM1468, which has yellow lupine as its natural host, and with *B. cepacia* BU61, a soil isolate (Taghavi *et al.*, 2005). Subsequent analysis of the endophytic community showed that neither VM1468 nor BU61 had succeeded in becoming successfully established at detectable numbers in this community. However, in both the presence and absence of toluene members of the endogenous endophytic community had via horizontal gene transfer of the pTOM-Bu61 plasmid successfully acquired the *tom* operon.

Despite the fact that both *B. cepacia* strains BU61 and VM1468 were able to transfer the pTOM-Bu61 plasmid to the endogenous microbial populations associated with poplar, major differences were observed (Taghavi *et al.*, 2005). Plants inoculated with the endophyte VM1468 suffered less from toluene toxicity and released less toluene into the environment, indicating that their microbial communities were better adapted to degrade toluene than plants inoculated with the soil isolate BU61. This observation was explained by the endophytic characteristics of strain VM1468: it was hypothesized that strain VM1468 could enter poplar where it was able to directly transfer pTOM-Bu61 to the endogenous endophytic community, despite the fact that the strain was unable to eventually establish itself. On the other hand, strain BU61 was unable to enter poplar as an endophyte. Therefore, BU61 could only act as a donor to transfer pTOM-Bu61 to bacteria present in the poplar rhizosphere. Since some rhizosphere bacteria can also colonize poplar as endophytes, the transfer of the *tom* operon on pTOM-Bu61 into the endophytic community with BU61 as donor strain will depend on the efficiency of subsequent endophytic colonization by the transconjugants, and will take more time than its direct transfer from an endophytic donor strain.

The observation of horizontal gene transfer of metabolic pathways, especially when they are encoded on mobile elements, opens the possibility to directly adapt the plant's endogenous endophytic population without the need of first selecting the appropriate endophytic microorganisms from the plant species of interest, and has several obvious advantages over the approach where an endophytic strain is optimized in a laboratory setup before being introduced into its host plant (Barac *et al.*, 2004): there is no need to isolate plant-specific endophytic bacteria, there is no need for genetic manipulation of isolated plant-specific endophytes, and there is no need to establish the endophytic inoculum in the plant's endogenous endophytic community as the genetic information will be transferred to many members of the endogenous endophytic population.

VI. CONCLUSIONS

The plant-endophyte association consists of very close interactions where plants provide nutrients and residency for bacteria, which in exchange can improve plant growth and health, either directly or indirectly. Several endophytic bacteria from poplar were shown to directly promote the growth and development of their host plant. However, other than increased above-ground biomass production, no information is presently available on how the presence of the endophytes affects C partitioning or metabolism. The idea that endophytic bacteria can affect the carbon partitioning in poplar deserves further attention, as it would open the possibility to improve the use of poplar for below-ground C sequestration. At the same time, the above-ground biomass could be harvested as a feedstock for biofuel production.

In order to minimize competition between food production and the production of biomass for biofuels, the cultivation of marginal soils for the sustainable production of poplar and other biofuel crops might provide an option. Marginal soils are often characterized by relatively low levels of nutrients and organic material, resulting in low water holding capacity. In addition, depending on the historical use of the sites, heavy metals or organic contaminants might be present. Plants grown on such marginal soils will most likely be stressed by a lack of nutrients and water, resulting in weakened plants that are more susceptible to diseases caused by pathogenic microorganisms. Poplar cuttings that were inoculated by selected plant growth promoting endophytic bacteria showed several characteristics that seem very promising to improve their establishment on marginal soils, including an increase of root formation that allows the plants to better access nutrients and water, and overall an increased growth and above-ground biomass production in comparison to non-inoculated poplar cuttings. In addition, we observed at several occasions that poplar plants that were inoculated with selected endophytic bacteria showed systemic drought resistance compared to non-inoculated control plants (Taghavi and van der Lelie, unpublished). The analysis of the genomes of four endophytic bacteria further revealed the presence of several mechanisms that might induce systemic resistance of their poplar host against drought stress and pathogens, as well as mechanisms that allow the endophytic bacteria to compete with pathogenic microorganisms. Understanding these mechanisms, which include antibiosis, competition for iron and the production of lytic enzymes such as chitinases, should result in improved biomass production by poplar trees.

In addition to their beneficial effects on plant growth, endophytic bacteria can contribute to an improved phytoremediation of organic contaminants by complementing or enhancing the metabolic properties of their host plant. While the improved phytodegradation of compounds such as BTEX and TCE by poplar and its endophytic bacteria has been well documented, other groundwater contaminants are not. One of these is methyl-t-butyl ether (MtBE), a major additive of gasoline. MtBE is extremely water-soluble and does not adhere well to soil, thus plumes of undetermined origin and several miles in length are not uncommon. Also, MtBE has been found in groundwater in states that do not use MtBE, perhaps the result of volatilization and deposition during rain events. Poplar readily takes up MtBE, but limited degradation has been observed. Instead, the majority of the compound is transpired unaltered, thus potentially increasing this spread of MtBE contamination. Application of endophytic bacteria, such as *M. populi* BJ001 that efficiently metabolizes MtBE, opens a promising route for the improved phytoremediation and improved containment of this contaminant.

Many endophytic bacteria are closely related to environmental and clinical isolates whose genomes have been or are in the process of being sequenced. Future genome annotation and comparative genomics of endophytic bacteria and phylogenetically closely related, non-endophytic microorganisms should result in the identification of the subset of genes necessary for a successful endophytic colonization of poplar. Understanding the interactions between endophytic bacteria and their host plant, facilitated by the published genome sequence of *Populus trichocarpa* (Tuskan *et al.*, 2006), should ultimately result in the design of strategies for improved poplar biomass production as a feedstock for biofuels, carbon sequestration, and bioremediation.

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REFERENCES

Ardon, O., Weizman, H., Libman, J., Shanzer, A., Chen, Y., and Hadar, Y. (1997. Iron uptake in Ustilago maydis: Studies with fluorescent ferrichrome analogues. *Microbiology-Uk* **143**: 3625–3631.

Arnold, C. W., Parfitt, D. G., and Kaltreider, M. 2007. Phytovolatilization of oxygenated gasoline-impacted groundwater at an underground storage tank site via conifers. *Int. J. Phytoremed.* **9**: 53–69.

Asghar, H. N., Zahir, Z. A., and Arshad, M. 2004. Screening rhizobacteria for improving the growth, yield, and oil content of canola (Brassica napus L.). *Aust. J. Agric. Res.* **55**: 187–194.

Ashbolt, N. J., and Inkerman, P. A. 1990. Acetic-acid bacterial biota of the pink sugar-cane mealybug, saccharococcus-sacchari, and its environs. *Appl. Environ. Microbiol.* **56**: 707–712.

Barac, T., Taghavi, S., Borremans, B., Provoost, A., Oeyen, L., Colpaert, J. V. et al. 2004. Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. *Nat Biotechno.l* **22**: 583–588.

Barac, T., Weyens, N., Oeyen, L., Taghavi, S., van der Lelie, D., Dubin, D.et al. 2009. Application of poplar and its associated microorganisms for the in situ remediation of a BTEX contaminated groundwater plume. *Int. J. Phytorem.*: in press.

Barka, E. A., Gognies, S., Nowak, J., Audran, J. C., and Belarbi, A. 2002. Inhibitory effect of endophyte bacteria on Botrytis cinerea and its influence to promote the grapevine growth. *Biological Control* **24**: 135–142.

Belimov, A. A., Hontzeas, N., Safronova, V. I., Demchinskaya, S. V., Piluzza, G., Bullitta, S., and Glick, B. R. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.). *Soil Biol. Biochem.* **37**: 241–250.

Benhamou, N., Belanger, R. R., and Paulitz, T. C. 1996a. Induction of differential host responses by Pseudomonas fluorescens in Ri T-DNA-transformed pea roots after challenge with Fusarium oxysporum f sp pisi and Pythium ultimum. *Phytopathology* **86**: 1174–1185.

Benhamou, N., Belanger, R.R., and Paulitz, T. C. 1996b. Pre-inoculation of Ri T-DNA-transformed pea roots with Pseudomonas fluorescens inhibits colonization by Pythium ultimum trow: An ultrastructural and cytochemical study. *Planta* **199**: 105–117.

Bent, E., Tuzun, S., Chanway, C. P., and Enebak, S. 2001. Alterations in plant growth and root hormone levels of lodgepole pines inoculated with rhizobacteria. *Can. J. Microbiol.* **47**: 793–800.

Bertagnolli, B. L., DalSoglio, F. K., and Sinclair, J. B. 1996. Extracellular enzyme profiles of the fungal pathogen Rhizoctonia solani isolate 2B-12 and of two antagonists, Bacillus megaterium strain B153–2-2 and Trichoderma harzianum isolate Th008 .1. Possible correlations with inhibition of growth and biocontrol. *Physiol. Molec. Plant Pathol.* **48**: 145–160.

Brimecombe, M. J., De Leij, F.A.A.M., and Lynch, J. M. 2007. Rhizodeposition and Microbial Populations. In *The Rhizosphere, Biochemistry and Organic Substances at the Soil-Plant Interface*. R. Pinton, Z. V., P. Nannipiere (eds.). Boca Raton, FL: CRC Press. pp. 73–109.

Brodmann, D., Schuller, A., Ludwig-Muller, J., Aeschbacher, R. A., Wiemken, A., Boller, T., and Wingler, A. 2002. Induction of trehalase in Arabidopsis plants infected with the trehalose-producing pathogen Plasmodiophora brassicae. *Molec. Plant-Microbe Interact.* **15**: 693–700.

Burken, J. G., Ma, X. M., Struckhoff, G. C., and Gilbertson, A. W. 2005. Volatile organic compound fate in phytoremediation applications: Natural and engineered systems. *Zeitschrift Fur Naturforschung C-a Journal of Biosciences* **60**: 208–215.

Burken, J. G., and Schnoor, J. L. 1996. Hybrid poplar tree phytoremediation of volatile organic compounds. *Abstracts Am. Chem. Soc.* **212**: 106–AGRO.

Buyer, J. S., and Leong, J. 1986. Iron transport-mediated antagonism between plant growth-promoting and plant-deleterious pseudomonas strains. *J. Biolog. Chem.* **261**: 791–794.

Buyer, J. S., Wright, J. M., and Leong, J. 1986. Structure of Pseudobactin A214, a siderophore from a bean-deleterious pseudomonas. *Biochemistry* **25**: 5492–5499.

Cankar, K., Kraigher, H., Ravnikar, M., and Rupnik, M. 2005. Bacterial endophytes from seeds of Norway spruce (Picea abies L.. Karst). *Fems Microbiol. Lett.* **244**: 341–345.

Chen, X. H., Koumoutsi, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I. *et al.* 2007. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium Bacillus amyloliquefaciens FZB42. *Nature Biotech.* **25**: 1007–1014.

Chilcott, G. S., and Hughes, K. T. 2000. Coupling of flagellar gene expression to flagellar assembly in Salmonella enterica serovar typhimurium and Escherichia coli. *Microbiol. Mol. Biol. Rev.* **64**: 694–708.

Cho, S. M., Kang, B. R., Han, S. H., Anderson, A. J., Park, J. Y., Lee, Y. H. et al. 2008. 2R,3R-butanediol, a bacterial volatile produced by

Pseudomonas chlororaphis O6, is involved in induction of systemic tolerance to drought in Arabidopsis thaliana. *Molec. Plant-Microbe Interact.* **21**: 1067–1075.

Cotter, P. A., Yuk, M. H., Mattoo, S., Akerley, B. J., Boschwitz, J., Relman, D. A., and Miller, J. F. 1998. Filamentous hemagglutinin of Bordetella bronchiseptica is required for efficient establishment of tracheal colonization. *Infection and Immunity* **66**: 5921–5929.

Cunningham, S. D., and Berti, W. R. 1993. Remediation of contaminated soils with green plants – an overview. *In Vitro Cellular & Developmental Biology-Plant* **29P**: 207–212.

Cunningham, S. D., Berti, W. R., and Huang, J.W.W. 1995. Phytoremediation of contaminated soils. *Trends Biotech.* **13**: 393–397.

de Salamone, I.E.G., Hynes, R. K., and Nelson, L. M. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J.Microbiol.* **47**: 404–411.

Dell'Amico, E., Cavalca, L., and Andreoni, V. 2005. Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. *Fems Microbiol. Ecol.* **52**: 153–162.

Dietz, A. C., and Schnoor, J. L. 2001. Advances in phytoremediation. *Environ. Health Perspect.* **109**: 163–168.

Doty, S. L. 2008. Enhancing phytoremediation through the use of transgenics and endophytes. *New Phytologist* **179**: 318–333.

Doty, S. L., Dosher, M. R., Singleton, G. L., Moore, A. L., Van Aken, B., Stettler, R. F. *et al.* 2005. Identification of an endophytic rhizobium in stems of Populus. *Symbiosis* **39**: 27–35.

Doucete, W. J., Bugbee, B., Hayhurst, S., Planen, W. A., Donney, D. C., Taffinder, S. A. N., and Edwards, R. 1998. Phytoremediation of dissolved phase trichloroethylene using mature vegetation. In *Bioremediation and Phytoremediation: Chlorinated and Recalcitrant Compounds*. Wickraamanayakee, G. B., and Hinchee, R. E. (eds). Columbus, Ohio: Battelle Press, pp. 251–256.

Dowling, D. N., and O'Gara, F. 1994. Metabolites of pseudomonas involved in the biocontrol of plant-disease. *Trends Biotech.* **12**: 133–141.

Dylan, T., Ielpi, L., Stanfield, S., Kashyap, L., Douglas, C., Yanofsky, M. et al. 1986. Rhizobium-meliloti genes required for nodule development are related to chromosomal virulence genes in agrobacterium-tumefaciens. *Proceed.Nat. Acad. Sci. U.S.A.* **83**: 4403–4407.

Ferrieri, A. P., Thorpe, M. R., and Ferrieri, R. A. 2006. Stimulating natural defenses in poplar clones (OP-367) increases plant metabolism of carbon tetrachloride. *Int. J. Phytoremediation* **8**: 233–243.

Ferrieri, R. A., Gray, D. W., Babst, B. A., Schueller, M.J ., Schlyer, D. J., Thorpe, M. R. et al. 2005. Use of carbon-11 in Populus shows that exogenous jasmonic acid increases biosynthesis of isoprene from recently fixed carbon. *Plant Cell Environ.* **28**: 591–602.

Franke, I. H., Fegan, M., Hayward, C., Leonard, G., and Sly, L. I. 2000. Molecular detection of Gluconacetobacter sacchari associated with the pink sugarcane mealybug Saccharicoccus sacchari (Cockerell) and the sugarcane leaf sheath microenvironment by FISH and PCR. *Fems Microbiol. Ecol.* **31**: 61–71.

Garg, A. K., Kim, J. K., Owens, T. G., Ranwala, A. P., Do Choi, Y., Kochian, L. V., and Wu, R. J. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proceed. Nat. Acad. Sci. USA* **99**: 15898–15903.

Germaine, K., Keogh, E., Garcia-Cabellos, G., Borremans, B., van der Lelie, D., Barac, T. *et al.* 2004. Colonisation of poplar trees by gfp expressing bacterial endophytes. *Fems Microbiol. Ecol.* **48**: 109–118.

Goddijn, O., and Smeekens, S. 1998. Sensing trehalose biosynthesis in plants. *Plant J.* **14**: 143–146.

Golubov, A., Neubauer, H., Nolting, C., Heesemann, J., and Rakin, A. 2004. Structural organization of the pFra virulence-associated plasmid of rhamnose-positive Yersinia pestis. *Infection and Immunity* **72**: 5613–5621.

Gomez, L. D., Baud, S., Gilday, A., Li, Y., and Graham, I. A. 2006. Delayed embryo development in the ARABIDOPSIS TREHALOSE-6-PHOSPHATE SYNTHASE 1 mutant is associated with altered cell wall structure, decreased cell division and starch accumulation. *Plant J.* **46**: 69–84.

Gordon, M., Choe, N., Duffy, J., Ekuan, G., Heilman, P., Muiznieks, I. et al. 1998. Phytoremediation of trichloroethylene with hybrid poplars. *Environ. Health Perspectives* **106**: 1001–1004.

Han, S. H., Lee, S. J., Moon, J. H., Park, K. H., Yang, K. Y., Cho, B. H. et al. 2006. GacS-dependent production of 2R, 3R-butanediol by Pseudomonas chlororaphis O6 is a major determinant for eliciting systemic resistance against Erwinia carotovora but not against Pseudomonas syringae pv. tabaci in tobacco. *Molec. Plant-Microbe Interact.* **19**: 924–930.

Henderson, I. R., Navarro-Garcia, F., and Nataro, J. P. 1998. The great escape: structure and function of the autotransporter proteins. *Trends Microbiol.* **6**: 370–378.

Hurek, T., Reinholdhurek, B., Vanmontagu, M., and Kellenberger, E. 1994. Root Colonization and Systemic Spreading of Azoarcus Sp Strain-Bh72 in Grasses. *J. Bacteriol.* **176**: 1913–1923.

Jakobi, M., Winkelmann, G., Kaiser, D., Kempler, C., Jung, G., Berg, G., and Bahl, H. 1996. Maltophilin: a new antifungal compound produced by Stenotrophomonas maltophilia R3089. *J Antibiot. (Tokyo)* **49**: 1101–1104.

James, E. K. 2000. Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res.* **65**: 197–209.

James, E. K., Reis, V. M., Olivares, F. L., Baldani, J. I., and Dobereiner, J. 1994. Infection of sugar-cane by the nitrogen-fixing bacterium acetobacter-diazotrophicus. *.J.Experimental Bot.* **45**: 757–766.

Jennings, D. B., Ehrenshaft, M., Pharr, D. M., and Williamson, J. D. 1998. Roles for mannitol and mannitol dehydrogenase in active oxygen-mediated plant defense. *Proceed. Nat. Acad. Sci. USA* **95**: 15129–15133.

Jennings, D. B., Daub, M. E., Pharr, D. M., and Williamson, J. D. 2002. Constitutive expression of a celery mannitol dehydrogenase in tobacco enhances resistance to the mannitol-secreting fungal pathogen Alternaria alternata. *Plant J.* **32**: 41–49.

Jeun, Y. C., Park, K. S., Kim, C. H., Fowler, W. D., and Kloepper, J. W. 2004. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. *Biological Control* **29**: 34–42.

Jurkevitch, E., Hadar, Y., Chen, Y., Libman, J., and Shanzer, A. 1992. Iron uptake and molecular recognition in pseudomonas-putida – receptor mapping with ferrichrome and its biomimetic analogs. *J. Bacteriol.* **174**: 78–83.

Kloepper, J. W., Ryu, C. M., and Zhang, S. A. 2004. Induced systemic resistance and promotion of plant growth by Bacillus spp. *Phytopathology* **94**: 1259–1266.

Kluepfel, D. A. 1993. The behavior and tracking of bacteria in the rhizosphere. *Annual Rev. Phytopathol.* **31**: 441–472.

Krechel, A., Faupel, A., Hallmann, J., Ulrich, A., and Berg, G. 2002. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode Meloidogyne incognita (Kofoid & White) Chitwood. *Can. J. Microbiol.* **48**: 772–786.

Lamb, T. G., Tonkyn, D. W., and Kluepfel, D. A. 1996. Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. *Can. J. Microbiol.* **42**: 1112–1120.

LeChevallier, M. W., Welch, N. J., and Smith, D. B. 1996. Full-scale studies of factors related to coliform regrowth in drinking water. *Appl. Environ. Microbiol.* **62**: 2201–2211.

Lehtola, M. J., Nissinen, T. K., Miettinen, I. T., Martikainen, P. J., and Vartiainen, T. 2004. Removal of soft deposits from the distribution system improves the drinking water quality. *Water Res.* **38**: 601–610.

Lerat, E., Daubin, V., and Moran, N.A. 2003. From gene trees to organismal phylogeny in prokaryotes: The case of the gamma-proteobacteria. *Plos Biol.* **1**: 101–109.

Lerat, E., Daubin, V., Ochman, H., and Moran, N. A. 2005. Evolutionary origins of genomic repertoires in bacteria. *Plos Biol.* **3**: 807–814.

Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E.R.B., Taghavi, S., Mergeay, M., and van der Lelie, D. 2002. Endophytic bacteria and their potential applications. *Critical Rev. Plant Sci.* **21**: 583–606.

Ma, X. M., and Burken, J .G. 2002. VOCs fate and partitioning in vegetation: Use of tree cores in groundwater analysis. *Environ. Sci. Technol.* **36**: 4663–4668.

Ma, X. M., and Burken, J. G. 2003. TCE diffusion to the atmosphere in phytoremediation applications. *Environ. Sci. Technol.* **37**: 2534–2539.

Mahaffee, W .F., and Kloepper, J. W. 1997a. Bacterial communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth–promoting rhizobacterium or its genetically modified derivative. *Can. J. Microbiol.* **43**: 344–353.

Mahaffee, W. F., and Kloepper, J. W. 1997b. Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (Cucumis sativus L.). *Microbial Ecol.* **34**: 210–223.

Mastretta, C., Barac, T., Vangronsveld, J., Newman, L., Taghavi, S., and Lelie, D.v.d. 2006. Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. *Biotech. Genetic Engineering Rev.* **23**: 175–207.

Mastretta, C., Taghavi, S., van der Lelie, D., Mengoni, A., Galardi, F., Gonnelli, C. et al. 2009. Endophytic bacteria from seeds of nicotiana tabacum can reduce cadmium phytotoxicity. *Int. J. Phytoremediation* **11**: 251–267.

Merckx, R., Vanginkel, J.H., Sinnaeve, J., and Cremers, A. 1986. Plant-induced changes in the rhizosphere of maize and wheat.1. Production and turnover of root-derived material in the rhizosphere of maize and wheat. *Plant and Soil* **96**: 85–93.

Messiha, N.A.S., van Diepeningen, A. D., Farag, N. S., Abdallah, S. A., Janse, J. D., and van Bruggen, A. H. C. 2007. Stenotrophomonas maltophilia: a new potential biocontrol agent of Ralstonia solanacearum, causal agent of potato brown rot. *Eur. J. Plant Pathol.* **118**: 211–225.

Monchy, S., Benotmane, M. A., Wattiez, R., van Aelst, S., Auquier, V. Borremans, B. Mergeay, M., Taghavi, S. van der Lelie, D., and Vallaeys, T. 2006. Proteomic analyses of the pMOL30 encoded copper resistance in Cupriavidus metallidurans strain CH34. *Microbiology* **152**: 1765–1776.

Muralidharan, N., Davis, L. C., and Erickson, L. E. 1995. Fate of volatile chlorinated organic compounds in a laboratory chamber with alfalfa plants. *Environ. Sci. Technol.* **29**: 2437–2444.

Newman, L. A., and Reynolds, C.M. 2005. Bacteria and phytoremediation: new uses for endophytic bacteria in plants. *Trends Biotechnol.* **23**: 6–8; discussion 8–9.

Newman, L. A., Strand, S. E., Choe, N., Duffy, J., Ekuan, G., Ruszaj, M. et al. 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environ. Sci. Tech.* **31**: 1062–1067.

Newman, L. A., Wang, X. P., Muiznieks, I. A., Ekuan, G., Ruszaj, M., Cortellucci, R. et al. 1999. Remediation of trichloroethylene in an artificial aquifer with trees: A controlled field study. *Environ. Sci. Tech.* **33**: 2257–2265.

O'Sullivan, D. J., and O'Gara, F. 1992. Traits of Fluorescent Pseudomonas Spp Involved in Suppression of Plant-Root Pathogens. *Microbiol. Rev.* **56**: 662–676.

Pan, M. J., Rademan, S., Kunert, K., and Hastings, J. W. 1997. Ultrastructural studies on the colonization of banana tissue and Fusarium oxysporum f. sp. cubense race 4 by the endophytic bacterium Burkholderia cepacia. *J. Phytopathol.-Phytopathologische Zeitschrift* **145**: 479–486.

Ping, L. Y., and Boland, W. 2004. Signals from the underground: bacterial volatiles promote growth in Arabidopsis. *Trends Plant Sci. Sci.* **9**: 263–266.

Porteous Moore, F., Barac, T., Borremans, B., Oeyen, L., Vangronsveld, J., van der Lelie, D. et al. 2006. Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterization of isolates with potential to enhance phytoremediation. *Syst. Appl. Microbiol.* **29**: 539–556.

QuadtHallmann, A., Benhamou, N., and Kloepper, J. W. 1997. Bacterial endophytes in cotton: Mechanisms of entering the plant. *Can. J. Microbiol.* **43**: 577–582.

QuadtHallmann, A., and Kloepper, J. W. 1996. Immunological detection and localization of the cotton endophyte Enterobacter asburiae JM22 in different plant species. *Can. J. Microbiol.* **42**: 1144–1154.

Ramon, M., and Rolland, F. 2007. Plant development: introducing trehalose metabolism. *Trends Plant Sci.* **12**: 185–188.

Ramos-Gonzalez, M. I., Campos, M. J., and Ramos, J. L. 2005. Analysis of Pseudomonas putida KT2440 gene expression in the maize rhizosphere: In vitro expression technology capture and identification of root-activated promoters. *J. Bacteriol.* **187**: 4033–4041.

Relman, D.A., Domenighini, M., Tuomanen, E., Rappuoli, R., and Falkow, S. 1989. Filamentous Hemagglutinin of Bordetella-Pertussis – Nucleotide Sequence and Crucial Role in Adherence. *Proceed. Nat. Acad. Sci. USA* **86**: 2637–2641.

Rubin, E., and Ramaswami, A. 2001. The potential for phytoremediation of MTBE. *Water Res.* **35**: 1348–1353.

Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B. et al. 2009. The versatility and adaptation of bacteria from the genus Stenotrophomonas. *Nature Rev. Microbiol.* **7**: 514–525.

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Pare, P. W., and Kloepper, J. W. 2003. Bacterial volatiles promote growth in Arabidopsis. *Proceed. Nat. Acad. Sci. USA* **100**: 4927–4932.

Ryu, C. M., Murphy, J. F., Mysore, K. S., and Kloepper, J. W. 2004. Plant growth-promoting rhizobacteria systemically protect Arabidopsis thaliana against Cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *Plant J.* **39**: 381–392.

Schnoor, J. L., Licht, L.A., Mccutcheon, S. C., Wolfe, N. L., and Carreira, L. H. 1995. Phytoremediation of organic and nutrient contaminants. *Environmental Science & Technology* **29**: A318–A323.

Schroder, P., Harvey, P. J., and Schwitzguebel, J. P. 2002. Prospects for the phytoremediation of organic pollutants in Europe. *Environ. Sci. Pollution Res.* **9**: 1–3.

Spencer, M., Ryu, C. M., Yang, K. Y., Kim, Y. C., Kloepper, J. W., and Anderson, A. J. 2003. Induced defence in tobacco by Pseudomonas chlororaphis strain O6 involves at least the ethylene pathway. *Physiolog. Molec. Plant Pathol.* **63**: 27–34.

Sprent, J. I., and Defaria, S. M. 1988. Mechanisms of infection of plants by nitrogen-fixing organisms. *Plant and Soil* **110**: 157–165.

Taghavi, S., Barac, T., Greenberg, B., Vangronsveld, J., and van der Lelie, D. 2005. Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. *Appl. Environ. Microbiol.* **71**: 8500–8505.

Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N. et al. 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar. *Appl. Environ. Microbiol.* **75**: 748–757.

Thorpe, M. R., Ferrieri, A. P., Herth, M. M., and Ferrieri, R. A. 2007. C-11-imaging: methyl jasmonate moves in both phloem and xylem, promotes transport of jasmonate, and of photoassimilate even after proton transport is decoupled. *Planta* **226**: 541–551.

Trapp, S., Zambrano, K. C., Kusk, K. O., and Karlson, U. 2000. A phytotoxicity test using transpiration of willows. *Arch Environ. Contamin. Toxicol.* **39**: 154–160.

Trapp, S., Köhler, A., Larsen, L. C., Zambrano, K. C., and Karlson, U. 2001. Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. *J. Soil Sediment* **1**: 71–76.

Tuskan, G. A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U. et al. 2006. The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). *Science* **313**: 1596–1604.

Ulrich, K., Stauber, T., and Ewald, D. 2008b. Paenibacillus – a predominant endophytic bacterium colonising tissue cultures of woody plants. *Plant Cell Tissue and Organ Culture* **93**: 347–351.

Ulrich, K., Ulrich, A., and Ewald, D. 2008a. Diversity of endophytic bacterial communities in poplar grown under field conditions. *Fems Microbiol. Ecol.* **63**: 169–180.

Van Aken B., Peres, C. M., Doty, S. L., Yoon, J. M., and Schnoor, J. L. 2004a. Methylobacterium populi sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (Populus deltoides x nigra DN34). *Int. J. Syst. Evol. Microbiol.* **54**: 1191–1196.

Van Aken B., J.M.Y., and Schnoor, J. L. 2004b. Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic Methylobacterium sp. associated with poplar tissues (Populus deltoides x nigra DN34). *Appl. Environ. Microbiol.* **70**: 508–517.

van der Lelie, D., Barac, T., Taghavi, S., and Vangronsveld, J. 2005. Response to Newman: New uses of endophytic bacteria to improve phytoremediation. *Trends Biotechnol* **23**: 8–9.

van der Lelie, D., Schwitzguebel, J. P., Glass, D. J., Vangronsveld, J., and Baker, A. 2001. Assessing phytoremediation's progress in the United States and Europe. *Environ. Sc.i Technol.* **35**: 446A–452A.

van Loon, L. C., Bakker, P.A.H.M., and Pieterse, C.M.J. 1998. Systemic resistance induced by rhizosphere bacteria. *Ann. Rev. Phytopathol.* **36**: 453–483.

Vroblesky, D. A., Nietch, C. T., and Morris, J. T. 1999. Chlorinated ethenes from groundwater in tree trunks. *Environ. Sci. Technol.* **33**: 510–515.

Weyens, N., Boulet, J., Adriaensen, D., Timmermans, J.-P., Prinsen, E., Van Oevelen, S., D'Haen, J., Smeets, K., Van der Lelie, D., Taghavi, S., and Vangronsveld, J. 2009. Colonization and plant growth promoting capacity of the endophyte Pseudomonas putida W619 after inoculation in hybrid poplar. *FEMS Microbiol. Ecol.* submitted for publication.

Wiehe, W., Hechtbuchholz, C., and Hoflich, G. 1994. Electron-microscopic investigations on root colonization of lupinus-albus and pisum-sativum with 2 associative plant-growth promoting rhizobacteria, pseudomonas-fluorescens and rhizobium-leguminosarum bv trifolii. *Symbiosis* **17**: 15–31.

Wightman, F., Rauthan, B. S. 1975. *Evidence for the Biosynthesis and Natural Occurrence of the Auxin, Phenylacetic Acid, in Shoots of Higher Plants*. Tokyo: 15–27: Hirokawa Publ, Inc.

Worley, M. J., Ching, K.H.L., and Heffron, F. 2000. Salmonella SsrB activates a global regulon of horizontally acquired genes. *Molec. Microbiol.* **36**: 749–761.

Zhang, S., Reddy, M. S., and Kloepper, J. W. 2004. Tobacco growth enhancement and blue mold disease protection by rhizobacteria: Relationship between plant growth promotion and systemic disease protection by PGPR strain 90–166. *Plant and Soil* **262**: 277–288.